Supporting information

Uncovering the relationship between the change in heat capacity for enzyme catalysis and vibrational frequency through isotope effect studies

Hannah BL Jones[†Δ], Rory M Crean[#†Δ], Christopher Matthews[†], Anna B Troya[†], Michael J Danson[†], Steven D Bull[‡], Vickery L Arcus[§*], Marc W Van der Kamp[%*] and Christopher R Pudney[†*]

†Department of Biology and Biochemistry, ‡Department of Chemistry, # Doctoral Training Centre in Sustainable Chemical Technologies, University of Bath, Bath BA2 7AY, United Kingdom

§School of Science, Faculty of Science and Engineering, University of Waikato, Hamilton 3240, New Zealand

% School of Biochemistry, University of Bristol, Biomedical Sciences building, University Walk, Bristol BS8 1TD, United Kingdom

*Christopher R Pudney, Department of Biology and Biochemistry, University of Bath, Bath, United Kingdom. c.r.pudney@bath.ac.uk.
*Marc W van der Kamp, School of Biochemistry, University of Bristol, Cantock's Close, Bristol BS8 1TS, United Kingdom. marc.vanderkamp@bristol.ac.uk.
*Vickery L Arcus, School of Science, Faculty of Science and Engineering, University of Waikato, Hamilton 3240, New Zealand. varcus@waikato.ac.nz.

Author Contributions

HBLJ and CM performed experimental work. RMC, MWK and ABT performed computational simulations and calculations. All authors discussed and interpreted data. The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. Δ, these authors contributed equally.

Index **Page**

Molecular dynamics simulations: Model setup, restraints and simulation details

The *ss*GDH crystal structure 2CDB[1] was prepared for simulation using scwrl4[2] to revert the T41A mutation and modeller[3] to model in the missing loop at positions 50-59, based on the coordinates of the surrounding residues in chain A. The same loop conformation was subsequently transferred to the other three chains. To avoid a clash with this loop in chain C, conformer B (assigned 50% occupancy) was chosen for His118. (If not mentioned, conformer A was chosen for other residues with multiple conformers defined, including for the glucose O6.) Asn/Gln flips and His tautomers and were selected based on optimal hydrogen bonding contacts through the AmberTools facility reduce, with His66 and His319 singly protonated on Nδ1, others on Nε2. The exception was His297, which was protonated on Nδ1: test simulations indicated that this tautomer better retained the crystallographically determined enzyme-ligand conformation structure. All ionizable residues (with exception of the Cys residues coordinating Zn^{2+}, see below) were modelled in their standard protonation states (in agreement with pK_a predictions by PropKa 3.1)[4]. To obtain similar starting points for the glucose and xylose complexes, the same protein starting model was used for the xylose complex (where xylose was placed based on alignment with 2CDC [1]).

The Amber16 suite of programs was used for periodic boundary simulations and analysis,[5] with the ff14SB force-field for protein atoms,[6] GLYCAM-06j for glucose/xylose,[7] parameters from Ryde *et al.* for NADP+,[8] TIP3P for water and ZAFF[9] for the Zn^{2+} coordinated by Cys93, Cys96, Cys99 and Cys107. These Cys residues were thus modelled as thiolates (CYM). For the Zn^{2+} directly adjacent to the substrate binding site, the default Amber ion parameters were used. To avoid coordination changes around the Zn^{2+} ion, the following one-sided harmonic restraints were applied if: 1) the His66 NE2–Zn^{2+} distance was larger than 2 Å (force constant 70 kcal mol^{-1} Å^{-2}); 2) the Asp42 OD2–Cys39 N distance was larger than 1.95 Å (force constant 70 kcal mol^{-1} Å^{-2}); 3) the Asp42 OD2–Zn2+ distance was smaller than 4.2 Å (force constant 100 kcal mol^{-1} Å^{-2}).

In addition to the water molecules present in the crystal structure, a rectangular box of water was added using the AmberTools facility tleap such that all protein atoms were at least 11.5 Å away from the edge of the box (closeness parameter of 0.9). 20 water molecules (at least 5 Å away from the protein) were replaced with Na^+ ions to neutralize the system. After minimization of solvent and hydrogens (100 steps steepest descent, 400 steps conjugate gradient), the full system was briefly minimized (400 steps) with positional restraints of 5 kcal mol^{-1} Å^{-2} on all Cα atoms. Maintaining these positional restraints on Cα atoms, velocities were assigned at 25 K and the system was heated to 300 K in 50 ps using Langevin temperature control in the NVT ensemble (with a 1 ps^{-1} collision frequency). Subsequently, 100 ps of equilibration was performed in the NPT ensemble at 1 atm, using the Berendsen barostat (1 ps pressure relaxation time) and Langevin dynamics for temperature control (5 ps^{-1} collision frequency). Thereafter, the positional restraints on Cα atoms were gradually removed in four consecutive 10 ps simulations under the same conditions (4, 3, 2, and 1 kcal mol^{-1} Å^{-2} as force constants). Production MD (without positional restraints) was then performed in the NPT ensemble at 1 atm and 300 K for 50 ns, saving coordinates every 10 ps. All MD simulations were performed with the default direct-space cut-off for non-bonded interactions and particle-mesh Ewald summation for long-range electrostatics, run with pmemd.cuda. Four independent simulations were run for each complex (GDH-glucose and GDH-xylose).

QM Cluster Model Calculations:* Model *Construction, pKa and KIE Calculations

The X-ray crystal structure of *ss*GDH in complex with glucose and $NADP^+$ (2CDB)[1] was used to create a 148 atom model of the active site. Truncations of the enzyme active site were made across non-polar bonds. All titratable residues included in the model were in their standard protonation state. The T41A mutation used to crystallise *ss*GDH was reverted in silico, with the rotamer (side chain alcohol group acting as a hydrogen bond acceptor to the alcohol group of the glucose C1) chosen based on its prevalence in our MD simulations. To preserve the overall structure of the active site, several atoms were fixed throughout the optimisation process and care was taken to ensure non-reacting groups stayed in the same local minima throughout the reaction.

All Calculations were performed using Gaussian16,[10] employing the M06-2X functional.[11] Geometry optimisations and frequency calculations were performed in vacuo with the 6-31G(d,p) basis set. All models were optimised on an ultrafine integration grid and under tight convergence criteria. Single point energies were obtained using the 6-311++G(2d,2p) basis set, with the surrounding protein environment accounted for using the SMD solvation model with a dielectric constant of 4.[12] Thermal corrections to the obtained energies were taken from the aforementioned frequency calculations, employing a scale factor of 0.97.[13] Contributions to tunnelling on the rate of reaction were estimated using Wigner's correction.[14]

In order to simulate the deprotonation of the Zn coordinated water to a Zn coordinated hydroxide by the bulk milieu (reactant to reactive intermediate), a proton on the Zn coordinated water (reactant model) was removed and the structure re-optimised, giving the reactive intermediate. During optimisation, the Zn coordinated hydroxide abstracted the sugar C1 alcohol group's proton, forming an alkoxide on the sugar. This therefore led us to propose the stepwise mechanism as seen in Scheme S1. To calculate the barrier for the transition from reactant to reactive intermediate, the experimentally determined barrier at 298 K (73.5 kJ mol^{-1}) was subtracted from the computationally determined hydride transfer barrier (32.6 kJ mol^{-1}), with the subsequent energy difference used to predict a pKa of 7.2.

All polar hydrogen atoms in the cluster model were considered deuterated when modelling the SKIE (see Figure S4). Heat capacities of activation (Table S2) were calculated from the differences in constant volume heat capacity for the reactive intermediate and transitions state models.

Tables

Table S1. Change in Hydride Transfer Frequency Upon Deuteration.

	Reactant Symmetric[a] $\bar{\upsilon}$ (cm^{-1})	Reactant Asymmetric[a] $\bar{\upsilon}$ (cm^{-1})	Reactive Intermediate $\bar{\upsilon}$ (cm^{-1})	Transition State $\bar{\upsilon}$ (cm^{-1})
glucose	3040.20	3047.13	2674.38	-576.79
D_1-glucose[c]	2236.61		1956.13	-523.85
D_5-glucose	3035.95	3047.03	2674.34	-574.96
D_7-glucose	2233.24	2239.84	1955.35	-523.10
D_{12}-glucose	2240.46	2237.05	1959.64	-521.65
D_5-glucose + D_20[b]	3036.01	3047.03	2677.49	-573.86
D_{12}-glucose + D_20[b]	2236.86	2240.33	1958.40	-520.76

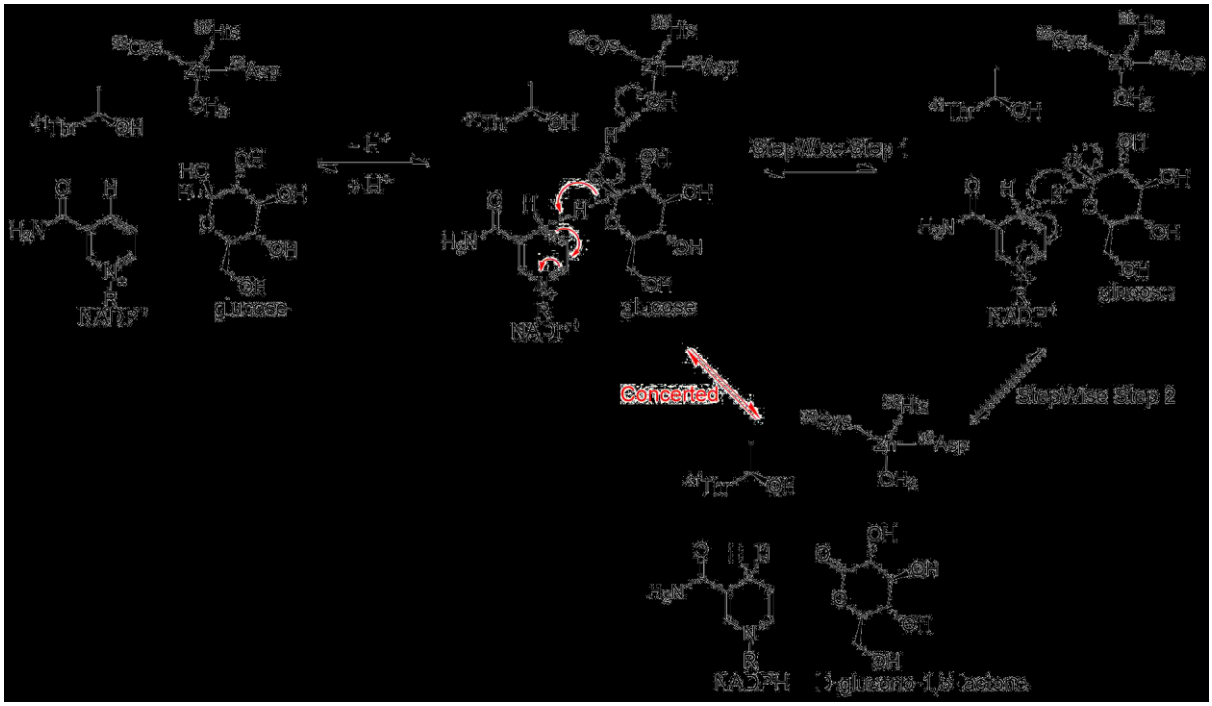
[a], Reactant symmetric and asymmetric stretches are coupled to additional C–H groups on glucose. [b], Mono-deuteration breaks symmetry leaving only one major C-H stretch, not significantly coupled to another other glucose C–H bond. [c], D_2O specifies a solvent kinetic isotope effect. Protons considered deuterated for SKIE calculations are indicated in Figure S4.

Table S2. Computationally Predicted Heat Capacity of Activation at Different Temperatures

Temperature (K)	glucose (j mol^{-1} K^{-1})	D_1-glucose (j mol^{-1} K^{-1})	D_5-glucose (j mol^{-1} K^{-1})	D_5-glucose + D_20 (j mol^{-1} K^{-1})	D_7-glucose[a] (j mol^{-1} K^{-1})	D_{12}-glucose[a] (j mol^{-1} K^{-1})	D_{12}-glucose + D_20 (j mol^{-1} K^{-1})
298	-0.255	0.351	-0.351	-0.690	0.309	0.222	-0.121
333	-0.142	0.502	-0.405	-0.890	0.468	0.205	-0.280
338	-0.134	0.510	-0.422	-0.924	0.472	0.184	-0.314
343	-0.134	0.506	-0.443	-0.957	0.477	0.167	-0.347
348	-0.134	0.510	-0.468	-0.999	0.477	0.142	-0.385
353	-0.134	0.506	-0.489	-1.032	0.472	0.117	-0.426
358	-0.142	0.497	-0.514	-1.070	0.464	0.088	-0.468
363	-0.146	0.489	-0.543	-1.112	0.460	0.063	-0.510

[a], D_7-glucose and D_{12}-glucose are not possible to measure experimentally and correspond to deuteration of only the alcohol groups on glucose (D_7), and deuteration of both the alcohol and aliphatic hydrogens on glucose (D_{12}).

Figures



Scheme S1. Two possible mechanisms for the oxidation of glucose by *ss*GDH. Proton abstraction and hydride transfer can occur in either a single concerted step (red and black curly arrows) or by a 2-step (black curly arrows only) mechanism, whereby a stable alkoxide intermediate is formed prior to hydride transfer.

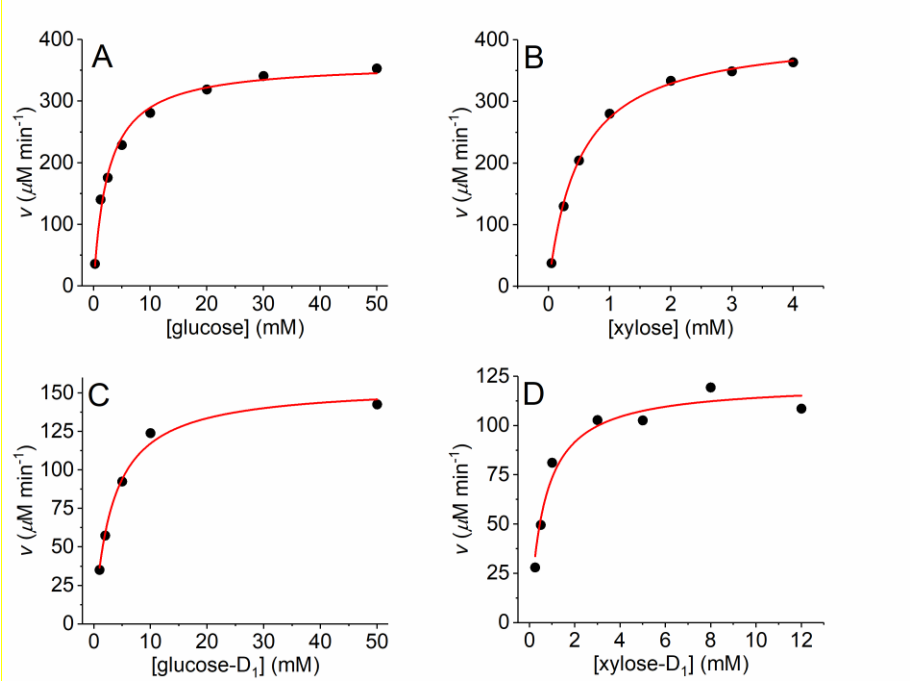


Figure S1. Michaelis-Menten plots for glucose **(A)**, xylose **(B)**, glucose-D_1 **(C)** and xylose-D_1 **(D)** with a saturating concentration of NADP. The solid red line is the fit to the Michaelis-Menten equation. Note that the enzyme concentrations are not identical for each plot and *k*cat values are given in the main text.

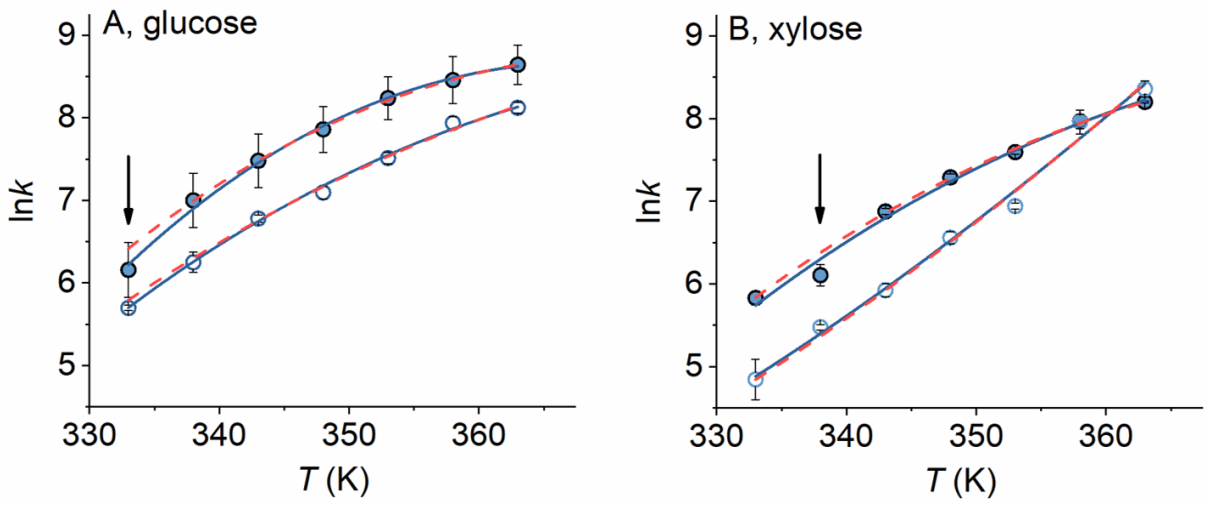


Figure S2. Alternative fitting of glucose (A) and xylose (B) temperature dependencies. Data corresponds to that shown in Figure 2A and 2B respectively. The solid lines are the fit to Eq 2 using all data points and the red dashed lines are the fit to Eq 2 omitting the 'outlier' data points shown with the black arrow. The corresponding $\Delta\Delta C_P^{\ddagger}$ values for the omitted data point fits are glucose $\Delta\Delta C_P^{\ddagger} = -1.44 \mp 1.33$ kJ mol^{-1} K^{-1} and xylose $\Delta\Delta C_P^{\ddagger} = -3.61 \mp 2.30$ kJ mol^{-1} K^{-1}, which is essentially the same as the fit including all data points.

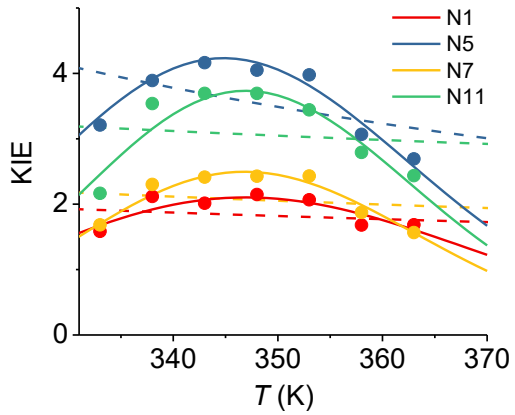


Figure S3. Comparison of the fit of the KIE data (Figure 4B) to either Eq 1 (dashed lines) or Eq 2 (solid lines). The curves are the numerical subtraction between the fits for the protiated and deuterated data sets and serves to show the trend in the data.

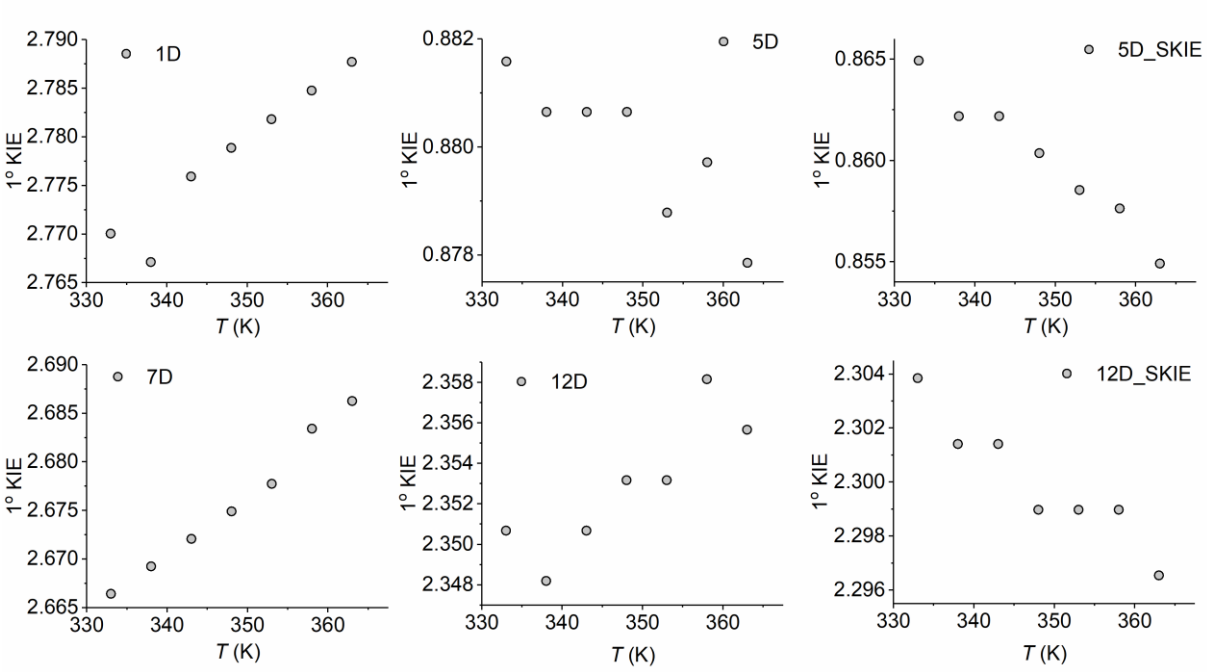


Figure S4. Temperature dependence of computationally calculated KIEs.

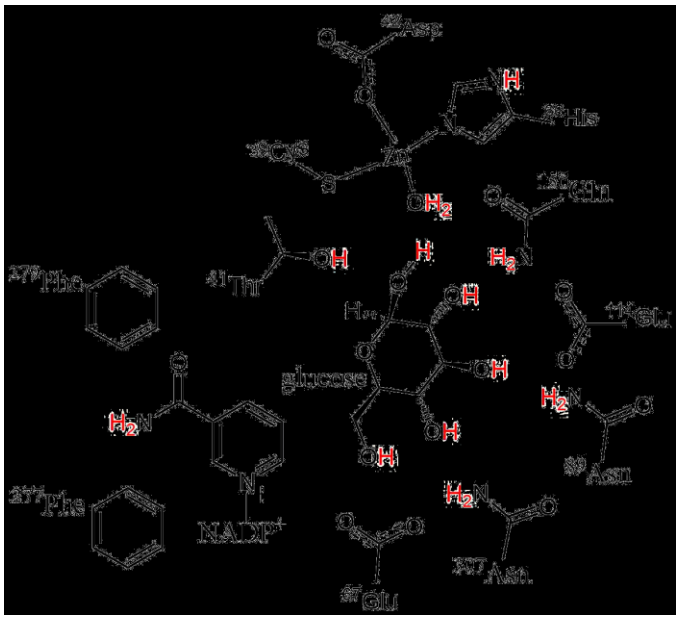


Figure S5. Molecules included in the QM cluster model of *ss*GDH. Red hydrogen atoms indicate those considered deuterated for the in silico measurement of the solvent kinetic isotope effect (SKIE).

Supporting References

(1) Milburn, C. C.; Lamble, H. J.; Theodossis, A.; Bull, S. D.; Hough, D. W.; Danson, M. J.; Taylor, G. L. The Structural Basis of Substrate Promiscuity in Glucose Dehydrogenase from the Hyperthermophilic Archaeon Sulfolobus Solfataricus. *J. Biol. Chem.* **2006**, *281*, 14796–14804.

(2) Krivov, G. G.; Shapovalov, M. V.; Dunbrack, R. L. Improved Prediction of Protein Side-Chain Conformations with SCWRL4. *Proteins Struct. Funct. Bioinforma.* **2009**, *77*, 778–795.

(3) Eswar, N.; Webb, B.; Marti-Renom, M. A.; Madhusudhan, M. S.; Eramian, D.; Shen, M.; Pieper, U.; Sali, A. Comparative Protein Structure Modeling Using Modeller. In *Current Protocols in Bioinformatics*; John Wiley & Sons, Inc.: Hoboken, NJ, USA, 2006; Vol. 24, p 5.6.1-5.6.30.

(4) Søndergaard, C. R.; Olsson, M. H. M.; Rostkowski, M.; Jensen, J. H. Improved Treatment of Ligands and Coupling Effects in Empirical Calculation and Rationalization of P K a Values. *J. Chem. Theory Comput.* **2011**, *7*, 2284–2295.

(5) Case, D. A.; Cerutti, D. S.; Cheatham, III, T. E.; Darden, T. A.; Duke, R. E.; Giese, T. J.; Gohlke, H.; Goetz, A. W.; Greene, D.; Homeyer, N,; Izadi, S.; Kovalenko, A.; Lee, T. S.; LeGrand, S.; Li, P.; Lin, C.; Liu, J.; Luchko, T.; Luo, R.; Mermelstein, D.; Merz, K. M.; Monard, G.; Nguyen, H.; Omelyan, I.; Onufriev, A.; Pan, F.; Qi, R.; Roe, D. R.; Roitberg, A.;Sagui, C.; Schott-Verdugo, S.; Shen, J.; Simmerling, C. L.; Smith, J.; Swails, J.; Walker, R. C.; Wang, J.; Wei, H.; Wolf, R. M.; Wu, X.; Xiao, L.; York D. M.; Kollman, P. A. AMBER 2016. University of Calafornia: San Francisco 2017.

(6) Maier, J. A.; Martinez, C.; Kasavajhala, K.; Wickstrom, L.; Hauser, K. E.; Simmerling, C. ff14SB: Improving the Accuracy of Protein Side Chain and Backbone Parameters from ff99SB. *J. Chem. Theory Comput.* **2015**, *11*, 3696–3713.

(7) Kirschner, K. N.; Yongye, A. B.; Tschampel, S. M.; González-Outeiriño, J.; Daniels, C. R.; Foley, B. L.; Woods, R. J. GLYCAM06: A Generalizable Biomolecular Force Field. Carbohydrates. *J. Comput. Chem.* **2008**, *29*, 622–655.

(8) Holmberg, N.; Ryde, U.; Bülow, L. Redesign of the Coenzyme Specificity in L-Lactate Dehydrogenase from Bacillus Stearothermophilus Using Site-Directed Mutagenesis and Media Engineering. *Protein Eng.* **1999**, *12*, 851–856.

(9) Peters, M. B.; Yang, Y.; Wang, B.; Füsti-Molnár, L.; Weaver, M. N.; Merz, K. M. Structural Survey of Zinc-Containing Proteins and Development of the Zinc AMBER Force Field (ZAFF). *J. Chem. Theory Comput.* **2010**, *6*, 2935–2947.

(10) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Petersson, G. A.; Nakatsuji, H.; Li, X.; Caricato, M.; Marenich, A. V.; Bloino, J.; Janesko, B. G.; Gomperts, R.; Mennucci, B.; Hratchian, H. P.; Ortiz, J. V.; Izmaylov, A. F.; Sonnenberg, J. L.; Williams-Young, D.; Ding, F.; Lipparini, F.; Egidi, F.; Goings, J.; Peng, B.; Petrone, A.; Henderson, T.; Ranasinghe, D.; Zakrzewski, V. G.; Gao, J.; Rega, N.; Zheng, G.; Liang, W.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Vreven, T.; Throssell, K.; Montgomery Jr., J. A.; Peralta, J. E.; Ogliaro, F.; Bearpark, M. J.; Heyd, J. J.; Brothers, E. N.; Kudin, K. N.; Staroverov, V. N.; Keith, T. A.; Kobayashi, R.; Normand, J.; Raghavachari, K.; Rendell, A. P.; Burant, J. C.; Iyengar, S. S.; Tomasi, J.; Cossi, M.; Millam, J. M.; Klene, M.; Adamo, C.; Cammi, R.; Ochterski, J. W.; Martin, R. L.; Morokuma, K.; Farkas, O.; Foresman, J. B.; Fox, D. J. Gaussian 16 Revision 16.A.03. 2016.

(11) Zhao, Y.; Truhlar, D. G. The M06 Suite of Density Functionals for Main Group Thermochemistry, Thermochemical Kinetics, Noncovalent Interactions, Excited States, and Transition Elements: Two New Functionals and Systematic Testing of Four M06-Class Functionals and 12 Other Functionals. *Theor. Chem. Acc.* **2008**, *120*, 215–241.

(12) Marenich, A. V.; Cramer, C. J.; Truhlar, D. G. Universal Solvation Model Based on Solute Electron Density and on a Continuum Model of the Solvent Defined by the Bulk Dielectric Constant and Atomic Surface Tensions. *J. Phys. Chem. B* **2009**, *113*, 6378–6396.

(13) Alecu, I. M.; Zheng, J.; Zhao, Y.; Truhlar, D. G. Computational Thermochemistry: Scale Factor Databases and Scale Factors for Vibrational Frequencies Obtained from Electronic Model Chemistries. *J. Chem. Theory Comput.* **2010**, *6*, 2872–2887.

(14) Wigner, E. Crossing of Potential Thresholds in Chemical Reactions. *Z. Phys. Chem.* **1932**, *B19*, 203–216.

Cartesian Coordinates of All Stationary Points Obtained

Reactant: Charge -1, Multiplicity 1

N	-5.72700	-0.86300	2.25800
N	-4.29200	-1.65700	0.81500
N	-3.18700	3.99300	-0.24300
N	-3.04800	1.31500	-2.64500
N	0.34200	5.82300	-0.96300
N	3.92800	-3.15100	-0.12100
N	6.39400	0.35000	-0.49800
C	-2.95300	-5.80200	0.06100
C	-1.56700	-4.32600	5.10600
C	-1.20800	-3.12100	4.23200
C	-1.12800	-1.81500	5.02600
C	-6.17400	1.46500	1.31100
C	-5.50900	0.14500	1.32500
C	-4.60700	-0.37300	0.44600
C	-4.97300	-1.94800	1.91700
C	-6.45700	-1.96800	-2.31700
C	-6.16900	-3.46500	-2.13400
C	-5.52700	4.71800	-0.53600
C	-4.20600	4.21000	-1.10800
C	-1.29800	6.60100	4.31500
C	-0.49100	5.31900	4.10000
C	-5.10000	1.38200	-3.97300
C	-3.91000	0.63000	-3.41300
C	2.64100	1.30300	-4.67700
C	1.47900	1.36300	-3.66600
C	7.85000	-1.85300	-3.37400
C	7.38000	-0.60500	-3.79700
C	6.95400	-2.91900	-3.21600
C	6.01900	-0.42300	-4.06700
C	5.59400	-2.73600	-3.49200
C	5.12700	-1.48900	-3.92100
C	6.74000	-2.20400	3.89000
C	5.35400	-2.18400	3.68900
C	7.50400	-1.05500	3.64300
C	4.72600	-1.01800	3.22900
C	6.87500	0.10700	3.18000
C	5.48900	0.12700	2.97400
C	2.72900	6.14900	-1.20200
C	1.31000	6.63500	-1.45900
C	0.35900	-0.49800	0.79800
C	-0.53600	0.31400	-0.15800
C	0.03600	1.74400	-0.23000
C	0.10900	2.35800	1.17200
C	0.83200	1.38700	2.14100
C	0.79600	1.82800	3.59900
C	4.44200	-4.41300	0.43500
C	4.71300	-2.04500	-0.13500
C	4.29000	-0.89000	-0.77500
C	3.03100	-0.89500	-1.38100
C	2.22900	-2.03600	-1.34900
C	2.69600	-3.15800	-0.70100
C	5.10000	0.38500	-0.91000
O	0.06100	-3.41000	3.61400
O	-6.99900	-4.28400	-2.53900
O	-5.03700	-3.79600	-1.58100
O	-4.09600	3.99000	-2.32100
O	-0.56500	4.44400	5.00900
O	0.12400	5.22200	2.99700
O	-3.79500	-0.59000	-3.65000

O	0.98300	2.46000	-3.33400
O	1.14400	0.21600	-3.18900
O	1.06600	7.66300	-2.09800
O	0.10400	-1.87700	0.86000
O	-0.64600	-0.27600	-1.44200
O	-0.72900	2.55200	-1.11800
O	0.82100	3.57400	1.08100
O	0.27000	0.05800	2.08700
O	-0.52400	1.94900	4.07500
O	4.58300	1.38700	-1.38200
O	-2.41100	-2.15600	-2.00300
S	-2.62100	-4.35200	1.14300
Zn	-3.54900	-3.05800	-0.55800
H	-2.10600	-5.88700	-0.62900
H	-3.88100	-5.73000	-0.53500
H	-1.97000	-3.02100	3.44600
H	0.16400	-2.81500	2.84900
H	-0.38200	-1.90300	5.82700
H	-0.84300	-0.97500	4.38300
H	-2.09700	-1.57400	5.48300
H	-5.93200	2.01700	0.38600
H	-5.85300	2.09700	2.15500
H	-6.32800	-0.79600	3.06900
H	-4.18200	0.08900	-0.43600
H	-4.93300	-2.89200	2.44100
H	-6.01300	-1.61400	-3.25900
H	-6.07800	-1.33600	-1.51400
H	-5.57500	4.59300	0.54700
H	-6.34300	4.14600	-0.99500
H	-2.29300	3.61500	-0.57700
H	-3.26300	4.20200	0.74200
H	-1.12800	7.33600	3.52500
H	-1.16300	7.07800	5.28700
H	-5.00600	2.47700	-4.00400
H	-5.99900	1.13700	-3.39300
H	-3.14200	2.32600	-2.55800
H	-2.18800	0.88300	-2.31000
H	2.64100	0.31100	-5.12900
H	3.56900	1.46200	-4.12200
H	8.07300	0.22300	-3.92600
H	7.32100	-3.89500	-2.90600
H	5.65200	0.54700	-4.38700
H	4.89800	-3.56600	-3.38700
H	4.07200	-1.34100	-4.13000
H	4.75600	-3.06500	3.91300
H	8.58500	-1.08000	3.76900
H	3.64700	-0.99600	3.09400
H	7.46000	1.00200	2.98600
H	5.00400	1.03400	2.62400
H	2.83100	5.76000	-0.18000
H	3.46700	6.94300	-1.35200
H	-0.62200	6.07500	-1.13500
H	0.52800	5.00000	-0.38700
H	-0.84700	-2.09700	0.81600
H	1.40400	-0.42300	0.45800
H	-1.54600	0.34100	0.27500
H	1.06400	1.68900	-0.62000
H	-0.91000	2.51800	1.55600
H	1.89000	1.33600	1.82000
H	0.06300	0.04000	-2.11500
H	-0.21200	2.63300	-1.96200
H	1.34600	1.06900	4.18300
H	1.33200	2.78300	3.68400

H	0.59100	4.14800	1.87100
H	-0.59700	2.86000	4.47700
H	4.76100	-5.06600	-0.38200
H	5.65500	-2.14100	0.38800
H	2.66100	-0.01700	-1.89500
H	1.23900	-2.02200	-1.79000
H	2.12000	-4.07100	-0.60200
H	6.96900	1.17200	-0.62400
H	6.88000	-0.49600	-0.21400
H	-2.92800	-1.65200	-2.69800
H	-0.72200	-4.54600	5.75900
H	-1.77100	-5.21900	4.50400
H	-2.44800	-4.11000	5.72300
H	-2.36100	6.31700	4.27600
H	-7.54100	-1.82800	-2.39000
H	-5.27700	1.00400	-4.98500
H	2.55300	2.08600	-5.43400
H	-7.26800	1.37600	1.36000
H	-5.65700	5.77500	-0.79200
H	-2.99900	-6.71700	0.66400
H	8.91400	-2.01200	-3.22000
H	7.22100	-3.10900	4.25600
H	3.65000	-4.91800	1.04100
H	5.28700	-4.22400	1.11900
H	2.95200	5.32300	-1.88900
H	-1.67000	-1.54300	-1.75900

Reactive Intermediate: Charge -2, Multiplicity 1

N	-5.80700	-0.99900	2.23200
N	-4.38900	-1.77500	0.76500
N	-3.29700	3.85600	-0.29800
N	-3.06700	1.60300	-2.79300
N	0.23300	5.91100	-0.84800
N	3.82500	-3.22200	-0.19000
N	6.29800	0.27500	-0.56400
C	-3.04000	-5.89600	-0.07100
C	-1.54800	-4.23000	5.05200
C	-1.22000	-3.04600	4.12500
C	-1.06400	-1.72600	4.88600
C	-6.26000	1.34300	1.33100
C	-5.59900	0.02000	1.30600
C	-4.70900	-0.49000	0.40800
C	-5.05400	-2.07900	1.86700
C	-6.56000	-2.02100	-2.35900
C	-6.05500	-3.46700	-2.17400
C	-5.62600	4.63200	-0.45800
C	-4.30200	4.24300	-1.11300
C	-1.37300	6.42700	4.40600
C	-0.40900	5.23500	4.23700
C	-5.21400	1.36100	-3.95900
C	-4.01500	0.74700	-3.22400
C	2.52300	1.30200	-4.70400
C	1.35400	1.37900	-3.70500
C	7.74100	-1.87200	-3.48800
C	7.27300	-0.62300	-3.90800
C	6.83500	-2.91900	-3.27100
C	5.90500	-0.41700	-4.11100
C	5.46600	-2.71200	-3.47800
C	5.00000	-1.46100	-3.89800
C	6.65900	-2.34600	3.81100
C	5.27100	-2.31600	3.61900
C	7.43200	-1.20700	3.54500
C	4.64600	-1.15300	3.15100
C	6.80400	-0.04600	3.07400
C	5.41800	-0.01800	2.87900
C	2.62500	6.08300	-1.14000
C	1.23300	6.64800	-1.38000
C	0.48900	-0.37200	0.58000
C	-0.50300	0.59200	-0.17300
C	0.12800	1.99100	-0.23700
C	0.33500	2.51800	1.18100
C	1.19300	1.49700	1.97300
C	1.31600	1.84900	3.45700
C	4.35600	-4.50700	0.29000
C	4.59500	-2.09500	-0.17700
C	4.14000	-0.91500	-0.73100
C	2.83700	-0.88300	-1.28500
C	2.07800	-2.06700	-1.33500
C	2.56900	-3.20400	-0.74700
C	4.96600	0.33900	-0.86900
O	-0.01200	-3.35800	3.41700
O	-6.81800	-4.41000	-2.43400
O	-4.82300	-3.61000	-1.79100
O	-4.19500	4.28600	-2.34600
O	-0.50400	4.30800	5.09100
O	0.38000	5.28100	3.24900

O	-3.93600	-0.47600	-3.05200
O	0.88300	2.49500	-3.38500
O	0.99800	0.24300	-3.23800
O	1.04500	7.68300	-2.03600
O	0.17700	-1.65300	0.61400
O	-0.87500	0.14600	-1.46600
O	-0.65500	2.88900	-1.03100
O	0.99600	3.77300	1.11300
O	0.68100	0.16200	1.90300
O	0.06600	1.84500	4.11200
O	4.45700	1.37700	-1.27600
O	-1.62200	-2.38000	-1.17700
S	-2.84000	-4.61900	1.22000
Zn	-3.40600	-3.11400	-0.50200
H	-2.17700	-5.93800	-0.74600
H	-3.95200	-5.79300	-0.68600
H	-2.03900	-2.94900	3.39800
H	0.02700	-2.80000	2.61300
H	-0.27600	-1.81800	5.64600
H	-0.78500	-0.90800	4.21200
H	-2.00000	-1.44800	5.39200
H	-6.00800	1.91600	0.42100
H	-5.93800	1.95100	2.19100
H	-6.39100	-0.94000	3.05400
H	-4.29900	-0.03900	-0.48600
H	-5.00000	-3.02900	2.37900
H	-6.07500	-1.60500	-3.25300
H	-6.24200	-1.41000	-1.51300
H	-5.63000	4.50100	0.62600
H	-6.42800	4.02300	-0.89200
H	-2.38600	3.54400	-0.66300
H	-3.41100	3.83700	0.70500
H	-1.42300	7.00100	3.48000
H	-1.07500	7.08800	5.21900
H	-5.39200	2.40900	-3.69500
H	-6.10600	0.77900	-3.70800
H	-3.18400	2.60800	-2.87700
H	-2.24100	1.22400	-2.32000
H	2.51700	0.32000	-5.17500
H	3.44800	1.44300	-4.14200
H	7.97300	0.19400	-4.07100
H	7.19600	-3.89300	-2.94700
H	5.53700	0.55800	-4.41600
H	4.75700	-3.51700	-3.30300
H	3.93600	-1.29000	-4.03300
H	4.66900	-3.19400	3.84500
H	8.51300	-1.23300	3.66400
H	3.56800	-1.12700	3.00600
H	7.39500	0.84200	2.85900
H	4.93500	0.88500	2.51300
H	2.73100	5.68200	-0.12000
H	3.38400	6.84900	-1.30300
H	-0.72100	6.16600	-1.06800
H	0.39700	5.05300	-0.31600
H	-0.96200	-2.20800	-0.42100
H	1.50200	-0.21200	0.04400
H	-1.42500	0.61500	0.42200
H	1.11600	1.91600	-0.71400
H	-0.63600	2.61900	1.68700
H	2.20700	1.52800	1.52700
H	-0.12300	0.24600	-2.13800
H	-0.26200	2.85700	-1.93900
H	1.97300	1.09200	3.91800

H	1.80600	2.82900	3.54300
H	0.80300	4.28800	1.94300
H	-0.10500	2.74800	4.49300
H	4.61100	-5.15400	-0.55400
H	5.56500	-2.20900	0.29000
H	2.49800	-0.01700	-1.83400
H	1.09700	-2.06900	-1.79100
H	2.00600	-4.12900	-0.69400
H	6.87200	1.10000	-0.67700
H	6.78700	-0.57500	-0.29800
H	-1.62600	-1.49200	-1.60700
H	-0.70100	-4.44400	5.70600
H	-1.74300	-5.13000	4.45700
H	-2.42800	-4.00700	5.66800
H	-2.37200	6.04800	4.65900
H	-7.64800	-2.01000	-2.46600
H	-5.04800	1.28600	-5.04000
H	2.45800	2.09600	-5.45500
H	-7.35600	1.26200	1.37400
H	-5.83800	5.67900	-0.69500
H	-3.09900	-6.88900	0.40100
H	8.80600	-2.03400	-3.34200
H	7.13600	-3.25300	4.17900
H	3.58200	-4.99600	0.93600
H	5.23900	-4.34000	0.92600
H	2.79600	5.25100	-1.83400

Transition State: Charge -2, Multiplicity 1

N	-5.80500	-0.91700	2.35600
N	-4.36200	-1.68200	0.90500
N	-3.26000	3.94700	-0.18800
N	-3.21600	1.46800	-2.55700
N	0.27900	5.93600	-0.80300
N	3.82800	-3.26900	-0.17400
N	6.38500	0.13200	-0.69000
C	-3.13000	-5.84000	0.02000
C	-1.48600	-4.26900	5.04000
C	-1.13400	-3.08300	4.12300
C	-0.94600	-1.77600	4.90000
C	-6.22900	1.43800	1.46900
C	-5.58100	0.10900	1.44200
C	-4.67500	-0.39400	0.55700
C	-5.04600	-1.99500	1.99400
C	-6.66100	-1.93700	-2.19300
C	-6.23900	-3.40900	-2.02400
C	-5.60000	4.71300	-0.34300
C	-4.29500	4.26000	-1.00000
C	-1.20900	6.47300	4.41100
C	-0.26100	5.27400	4.19600
C	-5.31600	1.42200	-3.84200
C	-4.10800	0.72000	-3.22800
C	2.40500	1.27900	-4.78500
C	1.26800	1.34300	-3.74200
C	7.61100	-1.95500	-3.67700
C	7.15600	-0.71600	-4.13900
C	6.68800	-2.94500	-3.31700
C	5.78300	-0.46500	-4.23900
C	5.31500	-2.69300	-3.42000
C	4.86100	-1.45200	-3.87800
C	6.69000	-2.38000	3.64700
C	5.29600	-2.30800	3.51200
C	7.48300	-1.26100	3.35700
C	4.68600	-1.12300	3.08000
C	6.87000	-0.07800	2.92100
C	5.47800	-0.00800	2.78500
C	2.64600	6.06700	-1.23400
C	1.24300	6.59700	-1.48500
C	0.70600	-0.45300	0.66000
C	-0.33700	0.46100	-0.10300
C	0.18700	1.89700	-0.25000
C	0.42700	2.46500	1.14600
C	1.43600	1.54900	1.88600
C	1.63000	1.95600	3.35100
C	4.31100	-4.56300	0.32200
C	4.59000	-2.13400	-0.11800
C	4.14400	-0.93100	-0.61200
C	2.78800	-0.83600	-1.11500
C	2.10800	-2.08000	-1.33400
C	2.59500	-3.23100	-0.80600
C	5.01900	0.27600	-0.78900
O	0.06800	-3.42200	3.42200
O	-7.03200	-4.29800	-2.36900
O	-5.05200	-3.62800	-1.55600
O	-4.22500	4.17300	-2.23400
O	-0.31600	4.34300	5.04700
O	0.47300	5.32000	3.16600
O	-4.00100	-0.51400	-3.35400
O	0.77500	2.44800	-3.41500
O	0.96100	0.20200	-3.25100

O	1.01600	7.51800	-2.28100
O	0.41900	-1.68700	0.76200
O	-0.74100	-0.07500	-1.34700
O	-0.71800	2.69800	-1.02300
O	0.95300	3.77700	1.03900
O	1.03900	0.16900	1.90100
O	0.42500	1.90100	4.08300
O	4.53000	1.36200	-1.09600
O	-2.38000	-2.08000	-1.82000
S	-2.82700	-4.51300	1.23800
Zn	-3.50900	-3.05200	-0.43100
H	-2.28100	-5.90400	-0.67200
H	-4.05200	-5.73100	-0.57300
H	-1.94900	-2.95600	3.39600
H	0.14300	-2.85600	2.62500
H	-0.16700	-1.89800	5.66500
H	-0.63600	-0.96100	4.23700
H	-1.87900	-1.47500	5.40100
H	-5.99000	2.00500	0.55600
H	-5.88000	2.04400	2.32400
H	-6.40700	-0.86700	3.16600
H	-4.24000	0.08300	-0.31100
H	-5.00700	-2.95300	2.49400
H	-6.19000	-1.53200	-3.10100
H	-6.31400	-1.32600	-1.35900
H	-5.58000	4.58200	0.73800
H	-6.42200	4.11300	-0.75800
H	-2.36700	3.58300	-0.55500
H	-3.33300	4.02500	0.81700
H	-1.28100	7.04500	3.47500
H	-0.87500	7.14100	5.19800
H	-5.25300	2.51200	-3.91900
H	-6.23000	1.15800	-3.30300
H	-3.30800	2.48100	-2.55000
H	-2.35600	1.05500	-2.18800
H	2.36400	0.28900	-5.24800
H	3.34000	1.38800	-4.23500
H	7.86900	0.06100	-4.40600
H	7.03800	-3.90800	-2.94900
H	5.42800	0.50500	-4.57200
H	4.59300	-3.44800	-3.12000
H	3.79600	-1.24400	-3.92400
H	4.67900	-3.17100	3.74800
H	8.56800	-1.30100	3.44700
H	3.60500	-1.06500	2.96900
H	7.47700	0.79400	2.68700
H	5.00500	0.90900	2.44400
H	2.77400	5.66600	-0.22100
H	3.41100	6.81800	-1.42800
H	-0.68500	6.06400	-1.07500
H	0.51600	5.17700	-0.15600
H	-1.65800	-1.45500	-1.52700
H	1.73800	-0.33300	-0.03800
H	-1.22600	0.45300	0.54100
H	1.14800	1.88600	-0.78200
H	-0.51600	2.47500	1.71400
H	2.40300	1.64500	1.35700
H	-0.05900	0.09900	-2.08700
H	-0.33000	2.72900	-1.93400
H	2.35600	1.25400	3.79200
H	2.06900	2.96400	3.37900
H	0.82400	4.26400	1.89800
H	0.20500	2.80200	4.44200

H	4.54500	-5.23800	-0.50900
H	5.57000	-2.26600	0.32700
H	2.53700	0.00400	-1.74700
H	1.15100	-2.08000	-1.83900
H	2.05600	-4.17000	-0.84600
H	6.97900	0.93700	-0.82900
H	6.84800	-0.72200	-0.39200
H	-2.92900	-1.55800	-2.46400
H	-0.62700	-4.48900	5.67600
H	-1.69100	-5.16000	4.43500
H	-2.35800	-4.04100	5.66600
H	-2.20200	6.10600	4.70100
H	-7.74900	-1.86900	-2.28600
H	-5.43600	1.00800	-4.85000
H	2.33100	2.08100	-5.52600
H	-7.32400	1.36800	1.53500
H	-5.78300	5.76400	-0.58700
H	-3.19000	-6.81000	0.53400
H	8.67600	-2.13500	-3.56400
H	7.15500	-3.30400	3.98700
H	3.52600	-5.01300	0.97200
H	5.20400	-4.40400	0.94400
H	2.80900	5.22800	-1.92500

Product: Charge -2, Multiplicity 1

N	-5.83300	-1.02400	2.24100
N	-4.37300	-1.76300	0.79300
N	-3.20900	3.95800	-0.21900
N	-3.10100	1.39400	-2.63000
N	0.27800	5.91300	-0.86800
N	4.07000	-3.09100	-0.16400
N	6.54400	0.41700	-0.50100
C	-3.06500	-5.89200	-0.03000
C	-1.51300	-4.17400	5.13800
C	-1.21200	-2.99800	4.20000
C	-1.05000	-1.67400	4.95400
C	-6.22700	1.36700	1.40300
C	-5.58900	0.02700	1.35800
C	-4.67400	-0.46400	0.47300
C	-5.07600	-2.10100	1.86200
C	-6.55900	-1.98200	-2.29800
C	-6.16100	-3.45700	-2.09700
C	-5.57200	4.65600	-0.37600
C	-4.26200	4.21700	-1.02800
C	-1.29600	6.40200	4.49000
C	-0.47900	5.13400	4.20600
C	-5.19000	1.39500	-3.89000
C	-3.98200	0.66600	-3.33200
C	2.54600	1.27800	-4.64900
C	1.34900	1.38100	-3.68600
C	7.73800	-1.95500	-3.43200
C	7.23800	-0.71100	-3.82900
C	6.85600	-3.02500	-3.22200
C	5.86300	-0.53400	-4.01200
C	5.48200	-2.84600	-3.40500
C	4.98400	-1.60000	-3.80000
C	6.67200	-2.33800	4.04900
C	5.29400	-2.31000	3.80000
C	7.48500	-1.27700	3.63000
C	4.72400	-1.22400	3.12500
C	6.91100	-0.19200	2.95500
C	5.53500	-0.16600	2.70400
C	2.68700	6.04500	-1.06600
C	1.31600	6.64900	-1.33000
C	-0.09400	-0.35600	0.81600
C	-0.67100	0.63900	-0.21100
C	0.09800	1.96000	-0.30900
C	0.28100	2.50100	1.10500
C	1.12600	1.50300	1.91900
C	1.12500	1.79000	3.41900
C	4.61100	-4.36700	0.27800
C	4.84600	-1.95800	-0.23400
C	4.39000	-0.76700	-0.70700
C	2.94100	-0.60600	-1.11900
C	2.24700	-1.93700	-1.23000
C	2.78900	-3.06400	-0.75900
C	5.23400	0.42100	-0.93100
O	-0.01400	-3.31200	3.47900
O	-6.98600	-4.34000	-2.38000
O	-4.96200	-3.68600	-1.66700
O	-4.19500	4.10300	-2.25800
O	-0.69800	4.15000	4.96800
O	0.29700	5.16800	3.20800
O	-3.86900	-0.56200	-3.51300
O	0.93300	2.52100	-3.35100
O	0.90200	0.26500	-3.25600

O	1.17000	7.71500	-1.94300
O	-0.38700	-1.53600	0.79500
O	-0.85800	0.02500	-1.46300
O	-0.62000	2.86600	-1.13800
O	0.94900	3.74300	1.06000
O	0.69900	0.10800	1.79000
O	-0.18100	1.73000	3.94600
O	4.78300	1.43300	-1.48400
O	-2.33000	-2.13400	-1.91700
S	-2.73200	-4.54700	1.16500
Zn	-3.43900	-3.11200	-0.51800
H	-2.28700	-5.90100	-0.80500
H	-4.04300	-5.83400	-0.53900
H	-2.04400	-2.91100	3.48700
H	-0.04500	-2.85000	2.62000
H	-0.23400	-1.75600	5.68500
H	-0.80800	-0.84900	4.27500
H	-1.97200	-1.41000	5.49200
H	-6.01200	1.92800	0.47800
H	-5.85400	1.97400	2.24300
H	-6.44900	-0.99100	3.04100
H	-4.22900	0.02800	-0.38200
H	-5.04400	-3.06800	2.34400
H	-6.07600	-1.58700	-3.20300
H	-6.24400	-1.36800	-1.45500
H	-5.58900	4.50800	0.70500
H	-6.39300	4.08200	-0.82400
H	-2.30700	3.64900	-0.60800
H	-3.26400	4.07400	0.78300
H	-1.03200	7.22600	3.82600
H	-1.25300	6.73800	5.52500
H	-5.10400	2.49100	-3.88600
H	-6.08300	1.12600	-3.31100
H	-3.19700	2.40500	-2.59100
H	-2.23300	0.96500	-2.29200
H	2.56200	0.29100	-5.11300
H	3.45600	1.41300	-4.04700
H	7.91700	0.12500	-3.98700
H	7.24200	-3.99400	-2.91200
H	5.47200	0.44100	-4.28600
H	4.78900	-3.66100	-3.21200
H	3.91400	-1.45100	-3.90500
H	4.66100	-3.13200	4.12600
H	8.55800	-1.31300	3.79800
H	3.65700	-1.21000	2.91600
H	7.53400	0.63200	2.61300
H	5.10100	0.66600	2.15700
H	2.77500	5.63600	-0.04900
H	3.46000	6.80300	-1.21200
H	-0.65900	6.20300	-1.11000
H	0.40700	5.02900	-0.37400
H	-1.64400	-1.48500	-1.61400
H	2.41400	0.03500	-0.39300
H	-1.67400	0.88100	0.17900
H	1.08600	1.79400	-0.75700
H	-0.70200	2.60600	1.59200
H	2.15400	1.54600	1.53400
H	-0.08900	0.19300	-2.15000
H	-0.16800	2.84000	-2.03000
H	1.76300	1.03500	3.90400
H	1.57500	2.78000	3.57800
H	0.74100	4.25000	1.89400
H	-0.38800	2.61600	4.35600

H	5.05400	-4.95200	-0.54200
H	5.86900	-2.10500	0.10000
H	2.87200	-0.06600	-2.06200
H	1.26100	-1.96400	-1.68000
H	2.27800	-4.02100	-0.78700
H	7.14700	1.17300	-0.78100
H	7.01200	-0.42600	-0.18400
H	-2.87000	-1.63200	-2.58600
H	-0.66100	-4.38000	5.78800
H	-1.70900	-5.08200	4.55500
H	-2.38900	-3.95800	5.76300
H	-2.35200	6.15400	4.30500
H	-7.64600	-1.91100	-2.41500
H	-5.35800	1.04000	-4.91100
H	2.51400	2.06900	-5.40400
H	-7.32000	1.30200	1.49800
H	-5.74000	5.71500	-0.59800
H	-3.01700	-6.86500	0.47900
H	8.80900	-2.11300	-3.33100
H	7.11100	-3.18700	4.57000
H	3.79100	-4.94900	0.75100
H	5.36300	-4.18600	1.06500
H	2.85700	5.21600	-1.76400